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EXAMINER	
STRZELECKA, TERESA E	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/607,077	ASHBY, MATTHEW	
	Examiner	Art Unit	
	TERESA E. STRZELECKA	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 December 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *45-50 and 57* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *45-50 and 57* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *Notice to Comply*.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 23, 2008 has been entered.

2. Claims 45-57 were previously pending, with claims 51-56 withdrawn from consideration. Applicant cancelled claims 51-56 and amended claims 45-49 and 57. Claims 45-50 and 57 are pending and will be examined.

3. Applicant is notified that claim 46 does not comply with the rule under 37 CFR 1.121, since the claim was amended, but the claim identifier was not changed to identify the claim as currently amended. In order to advance prosecution the amendment is considered, but Applicant is reminded that proper claim identifiers need to present in all submitted amendments.

4. Applicant's amendments overcame the objection to claim 57 and the rejection of claims 47-49 under 35 U.S.C. 102(b) as anticipated by Wikstrom et al. All other previously presented rejections are maintained for reasons given in the "Response to Arguments" section below.

5. The declaration under 37 CFR 1.132 filed December 23, 2008 is insufficient to overcome the rejection of claims 45-50 and 57 under 35 U.S.C. 112, first paragraph, enablement as set forth in the last Office action for reasons given in the "Response to Arguments" section below.

6. This office action contains new grounds for rejection.

Sequence Rules Compliance

7. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures. Applicant must comply with the requirements of the sequence rules (37 CFR 1.821 - 1.825) before the application can be examined under 35 U.S.C. §§ 131 and 132.

APPLICANT IS GIVEN time of response to this office action WITHIN WHICH TO COMPLY WITH THE SEQUENCE RULES, 37 C.F.R. §§ 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 C.F.R. § 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 C.F.R. § 1.136. In no case may an applicant extend the period for response beyond the six month statutory period. Direct the response to the undersigned. Applicant is requested to return a copy of the attached Notice to Comply with the response.

Figure 13, which outlines the SARD strategy, lists primers TX-009 and 1392R in the first step. Therefore, primer 1392R is essential for the best mode embodiment. However, no sequence of this primer was provided in the disclosure as filed.

Response to Arguments

8. Applicant's arguments filed December 23, 2008 have been fully considered but they are not persuasive.

The arguments from "Remarks" and from the declaration of Dr. Matthew Ashby are addressed jointly below.

A) Regarding the rejection of claims 45-50 and 57 under 35 U.S.C. 112, first paragraph, enablement, Applicant argues the following:

i) Example 3 provided evidence that nucleic acid tags obtained by the method of the present invention were useful in distinguishing the samples from each other, but the specification provides description of how to determine the correlation between the abundance of nucleic acid tags and environmental parameters.

ii) The declaration describes an experiment in which 1,664 non-identical tags from 21 soil samples were generated and the correlation was found between abundances of those tags and the abundance of aluminum and copper, as shown in exhibits A1 and A2, therefore this declaration shows that the application is enabled. Applicants further discuss other finding resulting from these experiments.

iii) The problems cited by Witzigenrode et al., such as presence of PCR inhibitors, differential amplification of templates, chimera formation can be mitigated by skilled worker. Further, the variation in a copy number of rRNA operons is not related to the claimed process, since it relates to the absolute number of microbial cells, whereas the claims are drawn to relative differences in the number of copies in one sample vs. other samples.

iv) With respect to Colbert et al., Applicants argue that observations made by Colbert et al. are not relevant to the claimed invention where the experiments were conducted over a short period of 5-7 days, whereas the "differences that take place in environmental microbial communities are a result of natural selection. They would, thus, be expected to occur over much longer time periods".

Regarding i) and ii), the methodology presented in Example 3 relies on the determination of 16S rRNA sequence tags from the bacteria present in the sample under analysis. Applicant provided a set of primers in Fig. 12 for such amplification, without any description of what these primers

amplify. Further, the tags are generated by ligation of very specific adapters and digestion with a set of very specific enzymes, namely, BpmI and AluI. Finally, none of these steps are in the claims, which do not even require obtaining 16S rRNA tags, adapter ligation or enzyme digests to form sets of tags. The results presented in the declaration were obtained at an unspecified time after the filing date of the instant application. Applicant is reminded that the disclosure needs to be enabling at the time of the invention. The claims are very broad, as the "environmental sample" can encompass a section of a colon with gut bacteria, a tree bark from a forest, a sample of sewage water, etc., each with corresponding technical challenges and unknown bacterial compositions.

Regarding iii), Applicant argues limitations which are not in the claims: there is nothing in the claims which requires determining a relative number of bacteria between the samples.

Regarding iv), Applicant again argues factors which have nothing to do with the claimed subject matter. The claims are drawn to any environmental sample, and there is nothing in the claims which requires samples collected or not over a certain period of time.

The rejection is maintained.

B) Regarding the rejection of claims 45-50 and 57 under 35 U.S.C. 102(b) as anticipated by Wikstrom et al., Applicant argues that Wikstrom et al. chooses a gene based on its function, rather than on its correlation to a parameter, since PAHs are substrates for catechol 2,3-dioxygenase. Wikstrom et al. does not recite inferring the presence of PAHs from the abundance of the 2,3-dioxygenease genes.

Applicant argues limitations which are not in the claims. The claims do not require choosing a gene based on its correlation to a parameter. Further, the catechol 2,3-dioxygenase is correlated to the concentration of PAHs in the samples. Further, Wikstrom et al. specifically teach inferring the

concentrations of PAHs from the presence of catechol 2,3-dioxygenase (Table 7, page 117, first paragraph).

The rejection is maintained.

Claim Rejections - 35 USC § 112, best mode

9. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claims 45-50 and 57 are rejected under 35 U.S.C. 112, first paragraph, because the best mode contemplated by the inventor has not been disclosed. Evidence of concealment of the best mode is based upon the fact that Applicant has not provided a sequence of the primer 1392R as shown in Fig. 13. As the amplification of the 16S rRNA is a prerequisite to the successful performance of the method as outlined in Fig. 13 and described in the examples, the lack of a sequence of this primer precludes even trying to repeat Applicant's steps.

Claim Rejections - 35 USC § 112, enablement

11. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

12. Claims 45-50 and 57 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention and breadth of claims

Claims 45-50 and 57 are broadly drawn to a culture-independent method of determining the abundance of an environmental parameter of interest by determining the abundance of at least one nucleic acid marker sequence, wherein the abundance of the nucleic acid marker sequence(s) correlates to the abundance of the environmental parameter, comprising the steps of:

a. providing an environmental sample containing a population of interest;

b. isolating genomic DNA from the environmental sample;

c. assaying the genomic DNA by utilizing at least one pair plurality of species-specific probes to at least one of the nucleic acid marker sequences as PCR primers to determine the abundance of at least one of the nucleic acid marker sequences in the genomic DNA isolated from the sample that shows a correlation to the parameter of interest; and

d. inferring the abundance of the parameter of interest based upon the abundance of at least one of the nucleic acid marker sequences in the genomic DNA isolated from the sample.

Claim 46 is drawn to using species-specific probes only, i.e., not as primers. Claim 50 is drawn to the environmental parameter being a subsurface oil or gas field.

However, as will be further discussed, there is no support in the specification and prior art for the methods. The invention is a class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." Mycogen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Fed. Cir. 2001).

Working Examples

The specification has no working examples of how to obtain a correlation between an abundance of a nucleic acid marker sequence and an abundance of any environmental parameter. In Example III on pages 44-46, Applicant examined a diversity of bacteria in two samples taken from Rocky Mountain Oilfield testing center by detecting a number of different 16S rRNA sequence tags in the genomic DNAs extracted from the samples. A total of 58 distinct tags were obtained for the Wy-1 sample, and 79 for the Wy-2 sample. Applicant concluded that 45% of tags obtained from the Wy-1 sample were not present in Wy-2, and 59% of tags present in Wy-2 were not present in Wy-1. There is no further investigation of how the number and/or type of the tags were possibly to be correlated with the presence of any parameter of interest, including oil. Applicant stated the following (page 46, lines 5-9):

"Thus, one cannot conclude that there are tags in these two samples that are indicators for various parameters associated with each sample. Nonetheless, a full-fledged analysis of these samples may provide such indicators."

Further, there are no working examples of detection of any other genetic markers and their correlation with various possible environmental parameters. Finally, Applicant's examples are based on the sequence of steps outlined in Fig. 13, which use specific primers, adapters and restriction enzymes to obtain a final product of 16S rRNA tags. However, the claims do not even require generation of rRNA tags, and Applicant did not provide a sequence of a primer 1392R which is necessary to reproduce Applicant's results.

Guidance in the Specification

The specification provides no evidence that the disclosed determination of 16S rRNA sequence tags in environmental samples would enable correlation of these tags with any environmental parameters. Further, the specification does not provide any guidance as to the use of any other genetic markers in correlating an abundance of such marker with an abundance of any environmental parameter. The guidance provided by the specification amounts to an invitation for the skilled artisan to try and follow the disclosed instructions to make and use the claimed invention.

The unpredictability of the art and the state of the prior art

The prior art provides a very strong evidence that it is not possible to reliably determine the abundance of different organisms in environmental samples, and, consequently, to correlate such abundance with the environmental parameters of interest. Witzingerode et al. (FEMS Microbiol. Rev., vol. 21, pp. 213-229, 1997; cited in the IDS) examined different stages of sample preparation for the detection of bacterial diversity by PCR amplification of 16S rRNA genes from environmental samples. Their conclusion (page 214, last paragraph):

"However, each physical, chemical and biological step involved in the molecular analysis of an environment is a source of bias which will lead to a distorted view of the 'real world. After 10 years of molecular ecological studies it seems necessary to summarize reported pitfalls of the molecular ecological approach which will most likely lead to an erroneous description of the diversity of a given ecological niche."

In particular, Witzingerode et al. teach that each of the processes leading to and including the PCR amplification step introduces an error in the final number of organisms detected, because

of losses of RNA or RNA fragmentation during sample lysis (page 215, last paragraph; page 216, paragraphs 1-3) and introduction of potential polymerase inhibitors (page 216, fourth paragraph) and problems during the PCR amplification process, such as inhibition of amplification by contaminants, non-uniform amplification due to different template properties and formation of PCR artefacts (page 217-220; page 221, paragraphs 1-5). Finally, since the number of rrn operons varies between different bacterial species and even within a single species the operon sequences vary due to insertion elements, the abundance of different bacterial species cannot be estimated from the abundance of different sequences, since in an unknown sample one does not know the numbers of rrn operons in each bacterial species present. As stated by Witzingerode et al. (page 222, third paragraph):

"In conclusion, 16S rRNA genes of some Bacteria and Archaea reflect the occurrence of inter- and intraspecific *rrn* operon heterogeneities. These differences can interfere with the analysis of 16S rDNA clone libraries or gel electrophoresis patterns derived from environmental ecosystems as it is not clear whether one 16S rDNA sequence represents a distinct organism or is just one representative gene of the entire 16S rRNA operon of an organism. Because it is likely that IVSs are introduced into 16S rRNA genes by lateral transfer their inclusion in phylogenetic analyses can lead to erroneous results."

Colbert et al. (Appl. Env. Microbiol., vol. 59, pp. 2056-2063, 1993) point to a different problem. They examined a relationship between the number of Pseudomonas putida PpG7 in soil amended with salicylate carbon source and the amount of the salicylate added as a function of time (Abstract; page 2057; Fig. 3). They found that increasing the concentration of salicylate initially increased the number of cells in the soil, but the bacterial population remained constant after the

salicylate has been consumed (Abstract; Fig. 3D-F; page 2060, last paragraph). Further, high salicylate concentrations lead to the suppression of metabolism and growth of the bacterial cells (Abstract; page 2061, second paragraph). Therefore, in case of bacterial species which depend on certain substances for growth, once the substances are exhausted their populations might not decrease, making the correlation between the presence of such substance and the number of bacteria impossible.

Quantity of Experimentation

The quantity of experimentation in this area is extremely large since there is significant number of parameters which would have to be studied to apply this technology to detection of any number of environmental parameters with any type of nucleic acid marker, which includes not only bacterial nucleic acid markers but any nucleic acid marker which can be found in the sample. Such parameters include choice of a number of different nucleic acid markers and testing those markers to determine whether the amount obtained by PCR or probe hybridization correlates with the presence of an oil deposit in the soil, for example. As the possible number of environmental parametrs is practically infinite, and the number of potential markers to study in the millions, this would require years of inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps.

Level of Skill in the Art

The level of skill in the art is deemed to be high.

Conclusion

In the instant case, as discussed above, in a highly unpredictable art where the abundance of a certain nucleic acid marker as detected by PCR or hybridization depends upon numerous known

and unknown parameters such as the method of RNA extraction, type of organisms present in the sample, time in which the sample was collected, etc., the factor of unpredictability weighs heavily in favor of undue experimentation. Further, the prior art and the specification provides insufficient guidance to overcome the art recognized problems in the use of the rRNA sequences for the determination of bacterial diversity. Thus given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of that art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the absence of a working example and the negative teachings in the prior art balanced only against the high skill level in the art, it is the position of the examiner that it would require undue experimentation for one of skill in the art to perform the method of the claim as broadly written.

Claim Interpretation

13. The term "environmental sample" has not been defined by Applicant, therefore it is interpreted as any sample.

14. The term "environmental parameter of interest" has not been defined by Applicant, therefore it is interpreted as any parameter.

15. The term "parameter of interest is surface oil or natural gas deposit" in interpreted as any parameter pertaining to oil or gas.

16. The terms "perfect correlation", a "high degree of correlation" and "moderate degree of correlation" have not been defined, therefore, the first two terms are treated as equivalent, and the third as any degree of correlation.

Claim Rejections - 35 USC § 102

17. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

18. Claims 45, 46 and 57 are rejected under 35 U.S.C. 102(b) as being anticipated by Wikstrom et al. (J. Biotechnol., vol. 52, pp. 107-120, 1996; cited in the previous office action).

Claims 45 and 46 are considered together in claim 45, since it is a species of claim 46.

Regarding claims 45 and 46, Wikstrom et al. teach a culture-independent method of determining the abundance of polycyclic aromatic hydrocarbons (PAHs) (=an environmental parameter of interest) by determining the abundance of an active catechol 2,3-dioxygenae gene (=at least one nucleic acid marker sequence), wherein the abundance of the nucleic acid marker sequence(s) correlates to the abundance of the environmental parameter, comprising the steps of:

a. providing an environmental sample containing a population of interest (page 108, last two paragraphs; page 109, first paragraph; page 111);

b. isolating genomic DNA from the environmental sample (page 109, last paragraph; page 110, paragraphs 1-5);

c. assaying the genomic DNA by utilizing at least one pair plurality of probes derived from genomic DNA specific to at least one of the nucleic acid marker sequences as PCR primers to determine the abundance of at least one of the nucleic acid marker sequences in the genomic DNA isolated from the sample that shows a correlation to the parameter of interest (page 110, last two paragraphs; page 112, first and second paragraphs; page 113 and Table 3; page 114; page 116, first paragraph; page 117, first paragraph); and

d. inferring the abundance of the parameter of interest based upon the abundance of at least one of the nucleic acid marker sequences in the genomic DNA isolated from the sample (page 117, first paragraph; Table 7; page 119, fourth paragraph).

Regarding claim 57, Wikstrom et al. teach choosing the gene on the basis of its function in using PAHs as substrates (page 108, second and third paragraph; page 119, paragraphs 2-4).

Claim Rejections - 35 USC § 103

19. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

20. Claims 47-49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wikstrom et al. (J. Biotechnol., vol. 52, pp. 107-120, 1996; cited in the previous office action) as evidenced by Clarke et al. (J. Nutr., vol. 120, pp. 218-224, 1990).

A) The teachings of Wikstrom et al. are described above. Regarding claims 47-49, Wikstrom et al. teach a correlation between the abundance of the catechol 2,3-dioxygenase DNA and the PAH concentration in the sample (Table 7; page 117, first paragraph, where there is a correlation between the levels of PAHs and the levels of the enzyme).

However, it was well known in the art at the time of the invention how to determine a correlation coefficient between two variables, for example, a level of gene expression and level of sugar, as shown by Clarke et al. on page 222, Fig. 4.

Therefore it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to represent the data of Wikstrom et al. in a form of correlation coefficients as customary in the art.

21. No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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